

AD _____

MIPR NO: 93MM3556

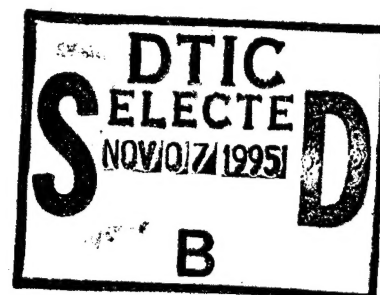
TITLE: The 3-D Structure of Staphylococcal Enterotoxins

PRINCIPAL INVESTIGATOR(S): M. Sax, Ph.D.
J. Pletcher, Ph.D.
S. Swaminathan, Ph.D.

CONTRACTING ORGANIZATION: Veterans Administration Medical Center
Pittsburgh, Pennsylvania 15240

REPORT DATE: October 15, 1995

TYPE OF REPORT: Annual



PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19951106 072

DTIC QUALITY INSPECTED 5

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 10/15/95	3. REPORT TYPE AND DATES COVERED Annual Report 10/1/94 - 9/30/95		
4. TITLE AND SUBTITLE The 3-D Structure of Staphylococcal Enterotoxins		5. FUNDING NUMBERS 93MM3556		
6. AUTHOR(S) M. Sax, J. Plether, S. Swaminathan				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Veterans Administration Medical Center Pittsburgh, Pennsylvania 15240		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick Frederick, MD 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) The aim of this research was to determine the crystal structures of staphylococcal enterotoxins. In the past year we solved the crystal structure of SEC2, and we modeled the 3-D structures of SEA and SEE based on our previous results for SEB and SEC2. As was predicted from the first structure of this family of proteins to have been determined & from their sequence homology, they share a common folding pattern, the SE fold. An important outcome of these studies was the identification of residues determining the V beta specifications of SEB, SEC, SEA and SEE. The crystal structure of a complex of SEB and 3-N-acetylneuramin-lactose was solved. The trisaccharide is the head group of glycosphingolipid, GM3.				
14. SUBJECT TERMS Staphylococcal enterotoxins, crystal structure, superantigen, pattern crystallography, xray diffraction		15. NUMBER OF PAGES 17		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet optical scanning requirements.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17 - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Accession For	
NTIS GRA&I	<input checked="checked" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

Martin Say
PI - Signature

10/13/95
Date

ij

TABLE OF CONTENTS

Front Cover	1
SF298	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Conclusion	7
References	7
Figure 1	8
Figure 2	9
Figure 3	10
Appendix	

STAPHYLOCOCCAL ENTEROTOXINS - ANNUAL REPORT

INTRODUCTION:

Staphylococcal enterotoxins secreted by *staphylococcus aureus* are both toxins and superantigens. The five distinct serotypes of s. enterotoxins, labeled SEA through SEE are divided into two groups based on their sequence homology. SEA, SED, and SEE form one group while SEB and SEC form the other. SEC itself could be further subdivided into SEC1-3 depending on the epitope variation. These toxins act as superantigens when presented by major histocompatibility complex class II (MHCII) molecules and induce massive proliferation of T cells bearing particular types of variable (V β) chains by forming a ternary complex of MHCII, superantigen and T-cell receptor (TCR). Unlike ordinary processed antigens, intact superantigens bind to a wide variety of MHCII molecules but with different degree of affinity. Binding studies of s. enterotoxins to MHCII molecules have shown that the binding mode of different s. enterotoxins to MHCII are different. While some of them require zinc to bind to MHCII, others do not. Similarly s. enterotoxins within the same group have different V β specificities though there may be some overlap. Though a model has been proposed for the formation of the ternary complex, recent results indicate that the model could be different depending on the type of s. enterotoxin. The crystal structure of MHCII has revealed that its structure is very similar to the MHC class I molecule. Crystal structure of soluble V β chain has been solved recently. The crystal structures of SEB, toxic shock syndrome toxin (TSST) , SEC2 and SEC3 have been determined. It is now possible to model a ternary complex for different serotypes of s. enterotoxin.

BODY:

The crystal structure of SEB was determined in this laboratory. The molecule consists of two domains (see figure 1); domain 1 is made up of a five stranded β cylinder with one end of the cylinder capped by a small α helix (α 3). Domain 2 mainly consists of two α helices with a five stranded twisted β sheet covering one side of both helices. Based on the topology of the molecule and on the results of mutational studies, regions and sites relevant for MHCII and TCR binding were proposed. The TCR binding site is located at the top of the molecule and is at the interface of the two domains . MHC binding site was proposed to be the entire front side of the molecule (on the side of α 5 helix). It was also proposed that in spite of their limited sequence homology all s. enterotoxins will possess a common folding pattern similar to SEB. This hypothesis has been proved to be right by the crystal structure determinations of TSST-1, SEC3 and SEC2 and this fold is now called the SE-fold. The three dimensional structure of SEB has helped scientists in designing

experiments for elucidating the mechanism of action of the enterotoxin and for identifying epitopes for developing vaccines (Jett et al., 1994).

The major aim of this project was to determine the crystal structure of all *s. enterotoxins*. In the past year we have crystallized SEC2 and determined its crystal structure. SEC2 was crystallized in two forms. Form 1 crystals are in space group P2₁ with cell dimensions $a = 43.43$, $b = 69.92$, $c = 42.22$ Å and $\beta = 90.1^\circ$. Form 2 crystals are in tetragonal space group P4₃2₁2 with cell dimensions $a = b = 42.98$ and $c = 289.92$ Å. Form 1 crystals were crystallized at pH 7.0 while form 2 at pH 6.5. We have now crystallized a third form at pH 8.0 using conditions similar to form 1. Form 3 crystals are also in space group P2₁ with cell dimensions $a = 43.3$, $b = 70.4$, $c = 42.2$ Å and $\beta = 90.3^\circ$. The crystal structure of form 1 crystals was solved at 2.7 Å resolution using a combination of the molecular replacement and the isomorphous replacement methods. As predicted earlier the folding is similar to SEB folding. On the basis of the homology, the three dimensional structures of SEA and SEE were modeled and a paper has been published (reprints enclosed) describing these results. An important outcome of this study was the identification of residues determining the V β specificity of these *s. enterotoxins*. Since form 1 crystals did not diffract to very high resolution, the crystal structure analysis of form 2 crystals which diffract to 2.0 Å was carried out. The diffraction data for these crystals with one very long dimension was collected with our newly acquired area detector system. The crystal structure was solved by the molecular replacement method using SEC2 structure from the monoclinic form as the starting model. An important discovery in this high resolution structure determination is the identification of a zinc binding site in SEC2 (Figure 2) which is different from that proposed for SEA or SEE (Figure 3). The structure has now been refined to 2.0 Å resolution using simulated annealing method. The final model now includes 235 residues, one zinc ion and 90 water molecules. The final R factor is 0.23 for 10969 reflections with $I > 1.0\sigma(I)$ in the resolution range 10 - 2.2 Å. The RMSD in bond lengths and bond angles are 0.016 Å and 2.3° respectively. It is now suggested that this zinc ion might play a role in the binding of SEC2 to MHCII. As seen in figures 2 and 3 the two zinc sites are different suggesting the mode of binding to MHCII may be different. A detailed paper on the three dimensional structure of SEC2 is in preparation.

As reported in the last annual report, domain 1 of SEB has been identified as oligomer or oligonucleotide binding site (OB fold). SEB has been shown to bind to glycosphingolipids in kidney cells. We have determined the crystal structure of SEB cocrystallized with lactose which is the head group of lactosylceramide. Lactose is bound to

SEB but not exactly at the same site as reported for other toxins possessing the OB-fold; it appears to act as a cross link between SEB molecules.

Crystal structure of SEB and 3'-N-acetylneuramin-lactose complex has also been solved. This trisaccharide is the head group of another glycosphingolipid, GM3. This structure reveals a different binding site for this trisaccharide than lactose which shows that while the OB-fold is general the binding site is specific for different sugars. The structure is being analyzed.

CONCLUSION:

1. Efforts are still being made to improve the quality of SED crystals. Crystallization of other *s. enterotoxins* will be continued.

2. The structure - function relationships in SEB which were described first by us (Swaminathan et al., 1992) were deduced from the 3 dimensional structure analysis of SEB and from the available mutational data. We now are extending the technique to other members of the SE family of proteins. The goal is to correlate structural differences with variations in the biological activities of the member proteins, in order to gain further precision in defining the stereochemical factors influencing their activities.

3. We were invited to write a review article on the structure of staphylococcal enterotoxins which will form a chapter in a book titled "Toxin Structures".

REFERENCES:

1. S. Swaminathan, W. Furey, J. Pletcher and M. Sax (1992). *Nature*, **359**, 801-806.
2. S. Swaminathan, W. Furey, J. Pletcher and M. Sax (1995). *Nature Structural Biology*, **2**, 680-686.
3. M. Jett, R. Neill, C. Welch, T. Boyle, E. Bernton, D. Hoover, G. Lowell, R. E. Hunt, S. Chatterjee and P. Gemski (1994). *Infection and Immunity*, **62**, 3408-3415.

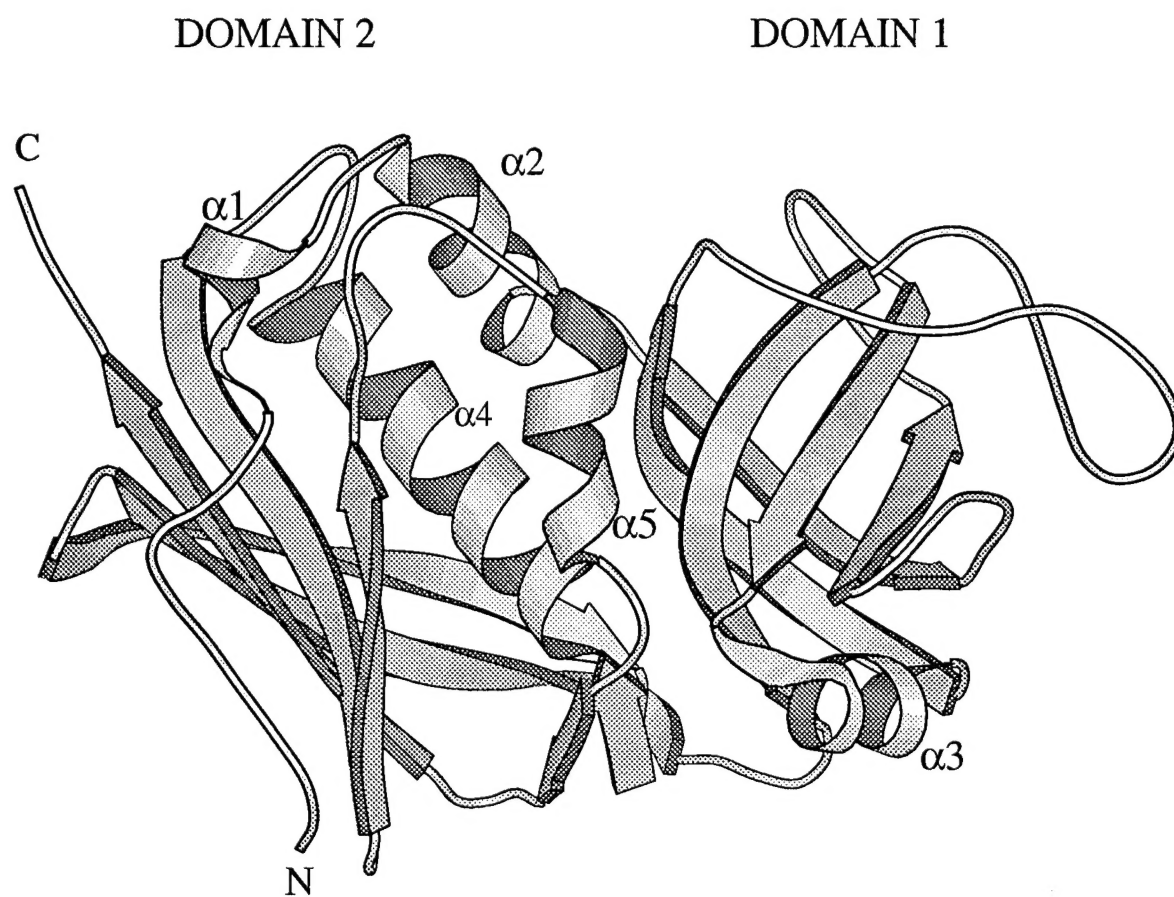


FIGURE 1: SEB

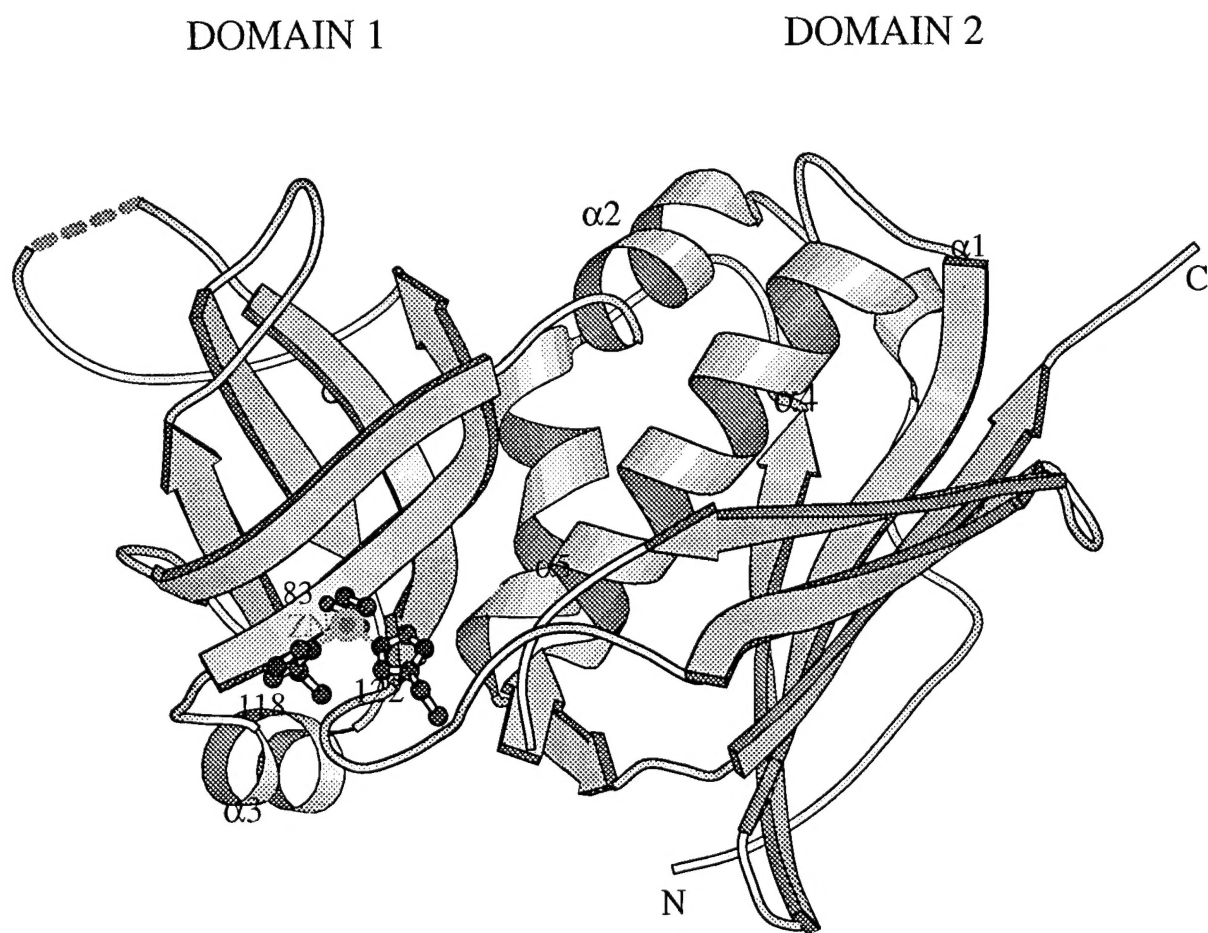


FIGURE 2: SEC2

